lipoproteins and the risk of coronary heart disease. The Framingham study. *Ann Intern Med* 1971;74:1-12.
9 Castelli WP. The triglyceride issue: a view from Framingham. *Am Heart J* 1986;112:432-7.
10 Bengtsson C, Blohmé G, Hallberg L, Hällstrom T, Isaksson B, Korsan-Bengtsen K, *et al.* The study of women in Gothenburg 1968-1969—a population study. General design, purpose and sampling results. *Acta Med Scand* 1973;193:311-8.
11 Bengtsson C, Hallberg L, Hällström T, Hultborn A, Isaksson B, Lennartsson J, *et al.* The population study of women in Göteborg 1974-75—the second phase of a longitudinal study. General design, purpose and sampling results. *Scand J Soc Med* 1978;6:49-54.
12 Bengtsson C, Gredmark T, Hallberg L, Hällström T, Isaksson B, Lapidus L, *et al.* The population study of women in Gothenburg 1980-81—the third phase of a longitudinal study. Comparison between participants and non-participants. *Scand J Soc Med* 1989;17:141-5.
13 Spri (Swedish Planning and Rationalization Institute). *Dödsorsak? Dödsorsaksstatistik som underlag för planering* [Cause of death? Statistics dealing with causes of death as a base for planning]. Stockholm: Spri, 1983. (Spri Report No 122: English summary.)
14 Levine JB, Zak B. Automated determination of serum total cholesterol. *Clin Chim Acta* 1964;10:381-4.
15 Lofland HB Jr. A semiautomated procedure for the determination of triglycerides in serum. *Anal Biochem* 1964;9:393-400.
16 Mantel N. Chi-square tests with one degree of freedom; extensions of the Mantel-Haenszel procedure. *Journal of the American Statistical Association* 1963;58:690-700.
17 Keys A, Menotti A, Aravanis C, Blackburn H, Djordević BS, Buzina R, *et al.* The seven countries study: 2,289 deaths in 15 years. *Prev Med* 1984;13:141-54.
18 Bradley PJ. Is obesity an advantageous adaptation? *Int J Obes* 1982;6:43-52.
19 Manson JA, Colditz GA, Stampfer MJ, Willett QW, Rosnea B, Monson R, *et al.* A prospective study of obesity and risk of coronary heart disease in women. *N Engl J Med* 1990;322:882-9.

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Association of restriction fragment length polymorphism in alcohol dehydrogenase 2 gene with alcohol induced liver damage

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Abstract

***Objective*—To investigate the role of genetically determined differences in the enzymes of alcohol metabolism in susceptibility to liver damage from misusing alcohol.**

***Design*—Use of pADH36 probe to study PVU II restriction length fragment polymorphism in alcohol dehydrogenase 2 gene in white alcohol misusers and controls.**

***Setting*—Teaching hospital referral centres for liver disease and alcohol misuse.**

***Subjects*—45 white alcohol misusers (38 with alcoholic liver disease) and 23 healthy controls.**

***Main outcome measures*—Alcohol misuse, the presence and severity of alcoholic liver disease, alcohol dependency, and family history of alcohol misuse.**

***Results*—A two allele polymorphism (A and B) was identified. In control subjects the allele frequencies were 85% for A and 15% for B compared with 37% and 63% respectively in alcohol misusers ($p < 0{\cdot}001$). B allele was significantly associated with severe liver damage ($p < 0{\cdot}05$) as well as alcohol dependency and family history of alcohol misuse compared with controls.**

***Conclusion*—Inherited variation in enzymes of ethanol metabolism may contribute to the pathogenesis of alcohol induced liver damage. This supports the presence of a genetic component in alcohol misuse.**

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Introduction

Although 10-15% of the population are classified as chronic alcohol misusers,[1] the incidence of alcohol related diseases varies considerably among people with comparable levels of intake. Alcoholic fatty liver is found in 90-100% of chronic misusers but only 10-20% subsequently develop cirrhosis.[2] A genetic effect in alcoholism was first suggested by studies of children of alcoholic parents adopted into non-alcoholic families,[3] and studies of concordance in twins have shown some genetic predisposition to alcohol induced cirrhosis.[4] Multiple environmental and genetic factors clearly influence drinking behaviour and the development of alcoholism,[5] and the inherited component in alcohol induced liver damage is almost certainly derived from several genes.

Genes influencing ethanol metabolism are likely to be the most important candidate genes for alcoholic liver disease. Studies in twins have shown that genetic factors account for most of the repeatable variation in ethanol metabolism between individuals.[6] In addition, dependent alcoholics undergoing detoxification show alterations in ethanol metabolism compared with misusers without signs of dependency or control subjects.[7] Acetaldehyde, the highly toxic product of ethanol metabolism, is thought to play an important part in alcohol induced liver damage and may also contribute to the pathogenesis of alcohol dependency.[8,9] It is therefore likely that alcohol dehydrogenase, which accounts for over 90% of ethanol metabolism in the liver and determines the rate of acetaldehyde formation, is implicated in genetic susceptibility to alcoholic liver disease.

Alcohol dehydrogenase shows considerable polymorphism. It has more than 20 different isoenzymes with greatly differing kinetic properties in vitro.[10,11] The enzyme is encoded by three gene loci, ADH_1, ADH_2, and ADH_3, which lie adjacent to each other on chromosome 4. Polymorphism is present only at the ADH_2 and ADH_3 loci.[12] We investigated the association between a genetic marker—a two-allele restriction fragment length polymorphism in the gene ADH_2—with historical features of alcoholic liver disease and clinical features of alcohol dependency in a white population.

Materials and methods

We studied 45 alcoholic patients and 23 non-alcoholic control subjects, all of whom were white. The patients were referrals to teaching hospital liver disease and alcohol misuse units, whereas the control subjects were research or laboratory staff recruited on a voluntary basis (table I). Although the average age of the controls was younger (mean 36 *v* 52 years), most of the alcohol misusers had established drinking habits by their mid-30s. All of the controls drank less than an average of 24 g of ethanol daily. All patients had consumed at least 80 g of ethanol daily (mean 146 (SE 9·8) g/day) for a minimum of two (mean 13·6 (1·8) years) and had come to medical attention because of the direct consequences of their alcohol misuse. Twenty one of the 45 patients showed clinical features of, and satisfied questionnaire criteria for, alcohol dependency and 19 had a family history of alcoholism, with at least one affected first degree relative.

Thirteen patients were referred for either detoxification or treatment of alcohol misuse, and 32 for treatment of acute alcoholic hepatitis or complications of cirrhosis (bleeding oesophageal varices, ascites, or

hepatic encephalopathy). Information on quantity, duration, and pattern of alcohol intake; severity of alcohol dependency; and family history was obtained by interview and completion of a questionnaire incorporating the criteria for alcoholism and dependency in the *Diagnostic and Statistical Manual of Mental Disorders, third edition, revised* and the severity of alcohol dependence questionnaire. Diagnostic liver biopsy specimens were obtained in 38 patients with persistently abnormal liver function. Six had fatty liver and the remaining 32 had histological features characteristic of advanced alcoholic liver disease (four had alcoholic hepatitis and fibrosis, 14 alcoholic hepatitis with cirrhosis, and 14 cirrhosis). All patients were negative for hepatitis B surface antigen and antibodies to smooth muscle and mitochondria.

ANALYSIS OF RESTRICTION FRAGMENT LENGTH POLYMORPHISM

Peripheral blood was stored at −70°C in 25 mM EDTA. Leucocyte DNA was prepared by standard phenol-chloroform extraction and precipitation in isopropanol and sodium acetate. DNA was digested with the restriction enzyme Pvu II, and the resulting fragments were separated by 0·7% agarose gel electrophoresis and transferred to nylon filters by Southern hybridisation.

We selected a 1·3 kb genomic DNA probe (pADH36) containing a polymorphic region of ADH_2.[13] A total of 50 ng of the probe was labelled with phosphorus-32 by the random oligonucleotide technique and hybridised with the filters. Before hybridisation the filters were incubated for 2 hours at 65°C in a mixture of six times strength saline sodium citrate, single strength Denhardt's buffer, 1% sodium dodeccylsulphate, 10 mM sodium phosphate, and 1 mM EDTA adjusted to pH 8, with 5% dextran sulphate and 25 μg/ml sonicated salmon DNA. This was followed by overnight hybridisation at 65°C. The filters were then washed to high stringency (in 0·1 strength saline sodium citrate and 0·1% sodium dodecylsulphate at 55°C) and autoradiographed.

The frequencies of the A and B alleles were calculated by adding the number of people with AA or BB genotype to half of those with the AB genotype. Statistical analyses were done by the χ^2 test.

Results

Analysis of the restriction fragments that hybridised to the pADH36 probe showed a two allele polymorphism. A and B alleles were denoted by hybridisation of the probe with 5·1/0·8 kb and 3·1/2·9 kb doublets respectively (figure). The number of subjects with homozygous and heterozygous ADH36 genotypes and the calculated frequencies of A and B alleles conformed to the model expected according to Hardy-Weinberg equilibrium. In control subjects, A and B allele frequencies were 85% and 15%, respectively, with only two patients homozygous for the B allele (table II). In contrast, the frequency of the B allele in the alcohol misuser group was 63%, significantly higher than in the control group ($p<0·001$). Histology was clearly associated with the B allele (table III). In patients with cirrhosis or alcoholic hepatitis with cirrhosis, the B allele frequencies were 86% and 61% respectively, higher than in the four patients with alcoholic hepatitis with fibrosis. The B allele was associated with cirrhosis ($p<0·05$) and was more prevalent in patients with cirrhosis without active hepatitis ($p<0·03$).

The association with the B allele remained strong when the 21 alcohol misusers with alcohol dependency and those with family history were considered separately (table III). B allele frequencies were 64% and 61% respectively ($p<0·001$ compared with controls).

TABLE I—*Characteristics of alcohol misusers and controls*

	Alcohol misusers (n=45)	Controls (n=23)
Mean (SE) age (years)	52·3 (1·7)	35 (2·6)
Sex (M/F)	26/19	13/10
No (%) in socioeconomic class:		
I	5 (11)	5 (22)
II	11 (25)	14 (61)
III	19 (42)	4 (17)
IV	9 (20)	0
V	1 (2)	0
Mean (SE) alcohol consumption (g/day)	146 (10)	<24
Mean (SE) duration of misuse (years)	13·6 (1·8)	
No (%) with alcohol dependency	21 (47)	
No (%) with family history	19 (42)	

TABLE II—*Genotypes and allele frequencies for pADH36 restriction fragment length polymorphism in controls and alcoholic patients*

	No of patients with genotype			Allele frequency (%)	
	AA	AB	BB	A	B
Controls (n=23)	18	3	2	85	15
Alcoholic patients (n=45)	7	19	19	37	63

$\chi^2=25·8$. $p<0·001$ (df=2).

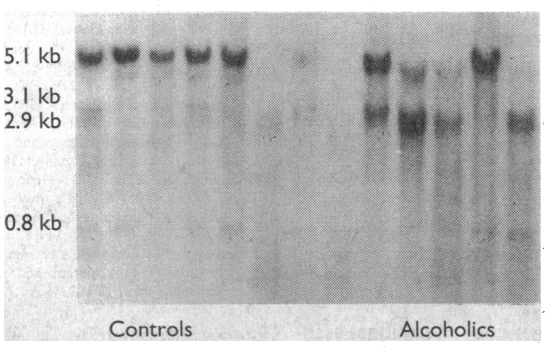
TABLE III—*Genotypes and allele frequencies of pADH36 restriction fragment length polymorphism in patients with severe alcoholic liver disease subdivided according to histology*

	No of patients with genotype			Allele frequency (%)	
	AA	AB	BB	A	B
Alcoholic hepatitis and fibrosis (n=4)	2	2	0	75	25
Cirrhosis (n=14)	1	2	11	14	86
Alcoholic hepatitis and cirrhosis (n=14)	2	7	5	39	61
All (n=32)	5	11	16	33	67

$\chi^2=11·3$, $p<0·025$ (df=4) for comparison between all three groups.
$\chi^2=6·1$, $p<0·05$ (df=2) comparing alcoholic hepatitis and fibrosis with all cirrhosis.
$\chi^2=8·1$, $p<0·025$ (df=2) comparing all alcoholic hepatitis with cirrhosis only.

Discussion

Patients with alcoholic liver disease and alcohol dependency both showed significant increases in the frequency of the B allele of this Pvu II fragment of ADH_2 compared with controls. However, the relation with severity of disease suggests that the B allele has a role in susceptibility to severe liver damage. These findings imply that an additional restriction site for the Pvu II enzyme occurs within ADH_2 in most alcoholic misusers but only a few controls. Analysis of the sequence of ADH_2 indicates that the additional restriction site is probably in a non-coding region, suggesting that this base alteration does not affect the function of alcohol dehydrogenase 2.[14] Therefore the most likely explanation is that the restriction fragment length



Autoradiograph of PvuII restricted leucocyte DNA from five control subjects and five alcoholic patients after Southern hybridisation with ^{32}P-labelled pADH36 probe

Clinical implications

- Only 10-20% of chronic alcohol misusers develop cirrhosis
- Genes encoding enzymes of alcohol metabolism are candidates for determining susceptibility to alcoholic liver disease and other alcohol related diseases
- This study identified a new genetic marker for susceptibility to alcoholic liver disease and alcohol dependency
- One allele of this alcohol dehydrogenase 2 polymorphism was associated with severe liver damage
- Inherited variations in enzymes of ethanol metabolism can contribute to the pathogenesis of alcohol induced liver damage

polymorphism is in linkage disequilibrium with either a polymorphism in an adjacent regulatory sequence, resulting in a change in ADH_2 expression, or a coding region of a neighbouring gene.

The ADH_2 locus is the most polymorphic of the alcohol dehydrogenase genes, with three alleles each encoding different polypeptides (β1, β2, and β3) that have greatly differing rates of ethanol oxidation in vitro.[10 11] Even though we have not determined these genotypes directly, published studies have shown that the β1 allele is predominant in white Europeans[15 16] and that the β2 allele is uncommon (2% or less) in this population. Since the probe we selected contained a coding sequence specific for the β2 allele, it is unlikely that the mutation site we detected corresponds to a structural change in the enzyme.

INFLUENCE OF GENOTYPES

Direct genotyping of the alcohol dehydrogenase genes from peripheral blood can be done using the polymerase chain reaction to amplify polymorphic areas of coding regions. Each of these polymorphic areas corresponds to a structural change in alcohol dehydrogenase and altered kinetic properties. Recent studies have attempted to show that particular alcohol dehydrogenase genotypes confer increased susceptibility to alcohol induced cirrhosis. A study from western France by Couzigou *et al* showed no differences in ADH_2 or ADH_3 allele frequencies between patients with cirrhosis and controls.[15] Day *et al*, however, identified a possible association between ADH_3 genotypes and cirrhosis in a population from north east England.[16] Further large scale studies are needed to clarify this issue.

Investigations into the genetic basis of susceptibility to complex multifactorial conditions such as alcoholic liver disease and alcohol dependency are difficult because multiple genes, each with varying levels of penetrance and differing interactions with the environment, are involved. A gene that determines ethanol metabolism can be assumed to have a large effect, but it is important to use accurate diagnostic criteria to select alcohol misusers for study, and to ensure that controls are racially matched. We therefore attempted to define our alcoholic patients carefully. In view of the increased female susceptibility to alcoholic liver disease[17] and known racial variations in ADH_2 gene frequency,[11 18] our samples were well matched with respect to sex and race. We did not match the two groups strictly according to age because it was a genetic study and an ethanol intake of 80 to 160 g per day is required over 10 to 20 years before chronic liver disease develops.[2] A questionnaire and measurements of haematological and biochemical markers were used to exclude heavy drinkers from the control population to maximise the chance of detecting a difference.

ALDEHYDE DEHYDROGENASE

Our results suggest that genetic alterations associated with the B allele of this ADH_2 restriction fragment length polymorphism may influence the rate of ethanol metabolism in the liver and hence the rate of formation of acetaldehyde. Acetaldehyde is rapidly oxidised, principally by mitochondrial aldehyde dehydrogenase. Reduced aldehyde dehydrogenase activity may also contribute to the pathogenesis of liver disease by reducing acetaldehyde clearance from the liver. Mitochondrial aldehyde dehydrogenase is inactive in at least 40% of Orientals because of a point mutation in the gene[19 20]; this has not been detected in white people.[16] People who are homozygous for this mutation rapidly accumulate acetaldehyde after drinking ethanol and the resulting symptoms of flushing, palpitations, and nausea cause aversion to further intake. Studies in Japanese have indicated that the presence of two copies of the point mutation protects against alcoholic liver disease,[21] although there is some evidence that susceptibility to liver damage is increased in the heterozygous state.[22] However, in whites it is likely that acetaldehyde induced liver damage is more dependent on the rate of acetaldehyde formation and is therefore influenced by alterations in alcohol dehydrogenase activity.

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1 Royal College of Physicians. *A great and growing evil: the medical consequences of alcohol abuse*. London: Tavistock, 1987.
2 Lelbach WK. Epidemiology of alcoholic liver disease. *Progress in Liver Disease* 1976;5:494-513.
3 Bohman M, Sigvardsson S, Cloninger CR. Maternal inheritance of alcohol abuse. *Arch Gen Psychiatry* 1981;38:965-9.
4 Hrubec Z, Omenn GS. Evidence of genetic predisposition to alcoholic cirrhosis and psychosis: twin concordances for alcoholism and its biological end points by zygosity among male veterans. *Alcohol Clin Exp Res* 1981;5:207-15.
5 Cloninger CR. Genetic epidemiology of alcoholism. In: Beigleter H, Cloninger CR, eds. *Genetics and biology of alcoholism*. New York: Cold Spring Harbor Laboratory Press, 1990:105-33. (Banbury Report series No 33.)
6 Martin NG, Perl J, Oakeshott JG, Gibson JB, Starmer GA, Wilks AV. A twin study of ethanol metabolism. *Behav Genet* 1984;15:93-109.
7 Peters TJ, Ward RJ, Rideout J, Lim CK. Blood acetaldehyde and ethanol levels in alcoholism. *Prog Clin Biol Res* 1987;241:215-30.
8 Sorrell MF, Tuma DJ. Hypothesis: alcoholic liver injury and the covalent binding of acetaldehyde. *Alcohol Clin Exp Res* 1988;9:306-9.
9 Collins MA, Nijm WP, Borge GF, Teas G, Goldfarb C. Dopamine-related tetrahydroisoquinolines: significant urinary excretion by alcoholics after alcohol consumption. *Science* 1979;206:1184-6.
10 Bosron WF, Magnes LJ, Li T-K. Kinetic and electrophoretic properties of native and recombined isoenzymes of human liver alcohol dehydrogenase. *Biochemistry* 1983;22:1852-7.
11 Bosron WF, Li T-K. Genetic polymorphism of human liver alcohol and aldehyde dehydrogenases and their relationship to alcohol metabolism and alcoholism. *Hepatology* 1986;6:502-10.
12 Hittle JB, Crabb DW. The molecular biology of alcohol dehydrogenase: implications for the control of alcohol metabolism. *J Lab Clin Med* 1988;112:7-15.
13 Smith M. Genetics of human alcohol and aldehyde dehydrogenases. In: Harris H, Hirschorn K, eds. *Advances in human genetics*. New York: Plenum Press, 1986:249-90.
14 Duester G, Smith M, Bilanchone V, Wesley Hatfield G. Molecular analysis of the human class I alcohol dehydrogenase gene family and nucleotide sequence of the gene encoding the β subunit. *J Biol Chem* 1986;261:2027-33.
15 Couzigou P, Fleury B, Groppi A, Cassaigne A, Begueret J, Iron A, French Group for Research on Alcohol and the Liver. Genotyping study of alcohol dehydrogenase class I polymorphism in French patients with alcoholic cirrhosis. *Alcohol Alcohol* 1990;25:623-6.
16 Day CP, Bashir R, James OFW, Bassendine M, Crabb DW, Thomasson HR, *et al*. Investigation of the role of polymorphisms at the alcohol and aldehyde dehydrogenase gene loci in genetic predisposition to alcohol-related end-organ damage. *Hepatology* 1991;14:798-801.
17 Saunders JB, Davis M, Williams R. Do women develop alcoholic liver disease more readily than men? *BMJ* 1981;282:1140-3.
18 Agarwal D, Goedde HW. Pharmacogenetics of alcohol dehydrogenase. *Pharmacol Ther* 1990;45:69-83.
19 Goedde HW, Agarwal DP. Polymorphism of aldehyde dehydrogenase and alcohol sensitivity. *Enzyme* 1987;37:29-44.
20 Crabb DW, Edenberg HJ, Bosron WF, Li T-K. Genotypes for aldehyde dehydrogenase deficiency and alcohol sensitivity: the inactive ALDH2[2] allele is dominant. *J Clin Invest* 1989;83:314-6.
21 Shibuya A, Yoshida A. Genotypes of alcohol-metabolising enzymes in Japanese with alcoholic liver disease: strong association of the usual caucasian-type aldehyde dehydrogenase gene (ALDH2[1]) with the disease. *Am J Hum Gen* 1988;43:744-8.
22 Enomoto N, Takase S, Yasukara M, Takada A. Alcoholic liver disease in heterozygotes of mutant and normal aldehyde dehydrogenase-2 genes. *Hepatology* 1991;13:1071-5.

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